A synthetic regulatory cascade for quantitative studies

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***Short Abstract* — Advancement in the field of synthetic, molecular, and quantitative biology allows the construction of synthetic gene regulatory networks using model regulatory proteins and DNA regulatory regions, and to measure their behavior quantitatively *in vivo*. We constructed a synthetic regulatory cascade that controls the expression of three different fluorescent reporters using the P_BAD /AraC, P_BAD /TetReCFP, P_LtetO-1 /LacI-mCherry, and P_TRC /eYFP systems. We also engineered an E. coli strain as a host for the joint functioning of these three regulatory proteins. Each of the components and the cascade as a whole is characterized by measuring the transfer function *in vivo* using fluorescent plate reader.**

THE proper regulation of transcription is crucial for all living cells. Regulation must be highly responsive because its environment can change dramatically and instantly. A given stress or change in food source can result in the repression or activation of one or more genes, which is often achieved by regulatory proteins interacting directly with a small effector molecules. Our understanding of prokaryotic gene regulation has been aided by the advancement in the field of molecular, structural and synthetic biology. This current knowledge allows us to construct novel synthetic regulatory networks using model regulatory proteins and their DNA regulatory regions [1-3]. Study of such simple synthetic networks is useful in understanding the operating and design principles of more complex genetic regulatory networks in living cells.

We constructed a synthetic regulatory cascade that controls the expression of three different fluorescent reporters using the P_BAD/AraC, P_BAD/TetReCFP, P_LtetO-1/LacI-mCherry, and P_TRC/eYFP systems. The concentration of the repressor molecules can be quantified by measuring the fluorescent intensity. Moreover, each of the steps in the cascades is controlled by inducer molecules. Currently, there is no *Escherichia coli* strains know to host these three regulatory proteins for the joint functioning. Therefore, we derived an *E. coli* strain from the parent strain BW27783 [4] by replacing the endogenous *lacI* with chloramphenicol resistance gene using phase λ Red recombinase system [5]. Furthermore, we characterized each of the components and the cascade as a whole by using corresponding inducer molecules *in vivo* in fluorescent plate reader.

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We developed an *E. coli* strain to work with three different inducible repressors and a synthetic cascade for quantitative studies.

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